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Fibre degradation of wheat straw by *Pleurotus erygnii* under low moisture conditions during solid state fermentation

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Running Title: Fungal decomposing wheat straw under low moisture

Significance and Impact of the Study

In this study, a white rot fungus, *P. erygnii*, effectively decomposed fibre under low moisture conditions when grown on wheat straw at similar levels under higher moisture conditions. However, the addition of wheat bran to wheat straw created a heterogenous system that appeared to allow *P. erygnii* to thrive under much lower moisture conditions although lower levels of fibre decomposition was obtained. These factors could influence the preparation of solid state fermentation.

Abstract

The application of solid state fermentation offers an alternative to conventional, submerged approaches for a variety of bioconversion processes, including animal feeds, biofuels and fungal bioproducts. Optimising solid state fermentation under low moisture conditions could significantly impact the proportion of dry biomass that

could be processed and improve the commercial viability of this approach, because of reduced input costs and higher yields of final products. *Pleurotus erygnii* that appeared to show tolerance to low moisture conditions was grown on saturated and desaturated wheat straw. *P. erygnii* showed insignificant fibre degradation although showed significantly lower biomass decomposition on desaturated wheat straw. Fibre decomposition by the fungus on wheat straw containing wheat bran showed marginally higher decomposition when saturated although there was no difference in biomass decomposition. The levels of delignification achieved were similar under different saturation conditions. It would appear that the fungus effectively decomposed fibre under low moisture conditions often resulting in lower biomass losses.

Keywords

moisture content · wheat straw · *Pleurotus* · evaporation · solid state fermentation

Introduction

Wood rot fungi that have a high tolerance to low moisture conditions with the ability to grow at much higher solid to liquid ratios could have useful applications in solid state fermentation of agricultural waste products (Wan and Li 2012). Some of the benefits would be lower energy requirements to regulate the temperature of the fermentation, which could significantly increase with larger quantities of waste and the opportunity to extract desirable products using organic solvents (Vamanu 2014). Most studies using agricultural waste substrates reveal that higher moisture contents support optimal growth of wood rot fungi (Asgher *et al*. 2006; Shi *et al*. 2008), leading to significantly higher levels of lignin degradation (Shi *et al*. 2008). Many of these studies have focused on the Basidiomycetes, *Phanerochaete chrysosporium*, *Lentinus edodes* and *Cereporiopsis subvermispora*, which showed a direct correlation between growth rate and water potential (Badham 1989; Boddy 1983; Wan and Li 2012). An evaluation of a wider range of Basidiomycetes has shown that each has a specific and different set of moisture conditions, which support optimum growth between 65-85% moisture condition and only one fungus grew optimally at a low moisture condition of 50-65% (Zadražil and Brunnert 1981). It has been suggested that fungi associated with dried fallen stems are most likely to exhibit higher tolerance to water stress conditions (Boddy 1983). Surprisingly, natural isolates of *Trametes* sp. and *Collybia sierraleonis* recovered from tropical regions did not show any increased level of tolerance to water stress

conditions compared with fungal strains recovered from temporal regions (Mswaka and Magan 1999; Singh 1989).

Wheat straw that has been pre-processed using some form of milling process contains vessels which, through capillary action, may draw water inside the plant matrix and the non-homogenous structure of this substrate (haphazardly arranged and different sized straw fragments) will ensure that there are spaces allowing gaseous exchange. However, wheat bran does not have the vessel structures but fine granular structure requires lower moisture conditions for higher enzymatic production as shown by the Ascomycete, *Trichoderma longibrachiatum* in producing xylanase (Azin *et al*. 2007), by the Ascomycete, *Aspergillus* sp. in producing cellulase (Vu *et al*. 2011) and by white rot fungus, *Pleurotus pulmonarius* in producing manganese peroxidase (Farani de Souza *et al*. 2006). Presumably, the granular composition of wheat bran causes the particles to become more closely associated, thus limiting the volume of space being formed between the bran particles that would allow gaseous exchange. Such high saturation conditions would have a negative influence on gas diffusion and perhaps other parameters such as enzyme activities, temperature regulation, and substrate inhibition (Gervais and Molin 2003). This is supported in a previous study showing that steady low levels of aeration are required in order to achieve significant rates of delignification (López *et al*. 2002).

The aim of this study was to determine the degradation characteristics of saturated and desaturated wheat straw by fungi, which showed quite high tolerance to low moisture conditions. The saturated wheat straw contains freely available water within the plants vessels allowing optimal fungal growth. In contrast, it is presumed that the desaturated wheat straw contains little free water except in the narrowest plant vessels. The availability of free water and bound water will have an impact on microbial utilization (Magan 2007). Fungi were selected based on a prior experiment revealing that *P. erygnii* showed quite high tolerance to low moisture conditions compared with other white rot fungi, while *P. pycnoporus* was somewhat tolerant (Baker, unpublished).

Results and discussion

The initial trial showed that *P. erygnii* and *G. tsugae* completely colonized the wheat straw, although *P. erygnii* showed greater changes in the fibre composition. Three fungi showed only partial colonization but showed fibre decomposition in the following decreasing order: *G. resinaceum*, *L. edodes*, and *P. sanguineus*. In a previous

82 study, it was shown that *L. edodes* was quite tolerant to water stress conditions when grown on wood rather than
83 glycerol amended medium (Badham 1989). In our study, *G. australe*, *P. ostreatus and C. subvermispora* failed
84 to colonize the wheat straw. This study was performed using smaller Microsacs with filter seams in closer
85 proximity to the substrate may have resulted in the significant decrease in moisture content of the wheat straw.
86 Therefore, only fungi which rapidly grew may have completely colonized the wheat straw.

87 The saturated and desaturated wheat straw substrate, containing mineral nutrients, was inoculated with similar
88 quantities at 41.2 ± 1.5 g and 48.8 ± 11.2 g of *P. erygnii* infected grain, respectively. It can be assumed that the
89 wheat straw that now has a lower moisture content because some of the water previously associated with wheat
90 straw was absorbed by wheat bran. It is possible that the addition of moist infected grain may allow moisture to
91 be translocated by the mycelium to as shown in a previous study in the fungal degradation of stacked wood
92 blocks (Stienen *et al*. 2014). The moisture contents of the saturated and the desaturated wheat straw at the start
93 of the experiment were significantly different ($P = 0.0004$) and the moisture content decreased significantly by
94 the end of the experiment in the saturated wheat straw ($P = 0.009$), whereas the moisture content in the
95 desaturated wheat straw remained unchanged (Table 1). The expectation would be that the moisture content
96 would increase as fungal degradation proceeds, but unlike wood, the fragments of wheat straw would allow for
97 evaporation that would offset the possible increase in moisture content. A direct comparison with the data in
98 another study revealed that these moisture contents lie within the optimal range (Zadražil and Brunnert 1981),
99 where the optimum may be midway (Saha *et al*. 2017). Fungal degradation significantly decreased the total dry
100 weight by 18.0 ± 3.0% in saturated wheat straw and by 11.0 ± 2.0% in desaturated wheat straw ($P = 0.03$). As
101 expected, the fibre composition in the saturated and desaturated experiments at the start was similar (Table 2)
102 and there were no significant differences in fibre composition between saturated and desaturated wheat straw at
103 the end of the experiment after fungal degradation. The fibre composition showed some significant changes
104 after fungal degradation, which differed under both saturation conditions compared with the undegraded wheat
105 straw at the start. It would be expected that after such a long incubation period where the fungus had
106 completely colonized the substrate that differences in fibre composition would be found, if there were any,
107 under the different treatments. It is uncertain whether a longer incubation would have shown differences.
108 Similar levels of delignification occurred under saturated and desaturated conditions and the total percentage of
109 lignin degraded was slightly higher (Table 3).

110 Similar quantities of *P. eryngii* infected grain were inoculated into wheat straw containing wheat bran
111 with 17.8 ± 2.1 g under saturated conditions and with 23.0 ± 4.5 g under desaturated conditions. The moisture

112 contents of saturated and desaturated wheat straw at the start of the experiment were significantly different (P =
113 0.007) and the moisture contents remained unchanged until the end of the experiment (Table 1). A direct
114 comparison with a previous study showed that the moisture contents of the desaturated wheat straw was just
115 below the lowest limit examined where degradation and delignification was restricted for most white rot fungi
116 (Zadražil and Brunnert 1981). The moisture contents of wheat straw containing wheat bran were significantly
117 lower compared with wheat straw containing mineral salts ($P < 0.01$) under both saturation conditions and
118 presented much lower moisture conditions. However, the optimal moisture conditions for fungal growth on
119 wheat bran are lower (Farani de Souza *et al*. 2006) compared with wheat straw and wheat bran may support the
120 initial stages of fungal growth. The addition of wheat bran to wheat straw at the start of the experiment
121 revealed that the proportion of non-fibre had significantly increased, whereas the proportion of cellulose had
122 decreased in comparison to the experiments containing only wheat straw (Table 2). The degradation in terms of
123 dry weights by *P. erygnii* in saturated and desaturated wheat straw were 14.1 ± 5.1% and 6.7 ± 5.6%, but these
124 were not significant different. Fungal degradation resulted in significant compositional changes in the straw,
125 causing an increase in the non fibre content ($P = 0.046$) and a decrease in the hemicellulose content ($P = 0.003$)
126 under saturated conditions compared with desaturated conditions (Table 2). Although a constant amount of
127 wheat bran (72 g) was added to 250 g of wet wheat straw (saturated or desaturated), wheat bran formed 50.9 ±
128 2.8% of the total dry weight under saturated conditions and 37.4 ± 0.4% of the total dry weight under
129 desaturated conditions. Therefore, the proportion of wheat bran associated with saturated wheat straw was
130 significantly greater ($P = 1x10^{-6}$) and growth of *P. erygnii* appeared to be more favourable under saturated
131 conditions. The addition of wheat bran appeared to have lowered the extent of delignification but this was not
132 significantly different under saturated or desaturated conditions (Table 3). It is uncertain whether the lower
133 levels of delignification were caused by using an easily accessible substrate, wheat bran, or by using lower
134 quantities of grain inoculums. The total percentage of lignin degraded was higher in comparison to the
135 percentage of delignification due to the higher biomass losses. *P. erygnii* appeared to cause similar levels of
136 degradation under these water stress conditions compared with more saturated conditions, which is in contrast
137 with most white rot fungi grown under slightly higher moisture conditions of 50% (Zadražil and Brunnert
138 1981).

139 The results confirmed those obtained from an initial trail showing that *P. erygnii* caused similar
140 levels of fibre decomposition under both saturation conditions. The addition of dry wheat bran reduced the
141 moisture content further but this did not affect fibre decomposition by either fungi even under the much lower

moisture conditions. The growth of fungi under lower moisture conditions would facilitate the extraction of compounds that require organic solvents and improve the operating conditions for solid-state fermentation.

Materials and methods

Preparation of fungal inoculum

A variety of fungi: *Ganoderma resinaceum* GR, *Ganoderma tsugae* XHMF, *Ganoderma australe* GA1, *Lentinus edodes* CYN, *Pleurotus erygnii*, *Pycnoporus sanguineus* V and *Pleurotus ostreatus* Pox K from the Bangor University culture collection and *Ceriporiopsis subvermispora* D98698 from the VTT (Finland) culture collection were grown on desaturated wheat containing mineral solution in an environmentally controlled room during an initial trial. An initial trial was performed to determine which fungi grew under low moisture conditions using Microsacs (49 cm x 22 cm with 5 filter seams – 4 on one side and 1 on the other). This experiment was repeated in triplicate based on the trial using *P. erygnii* which grew well under low moisture conditions. These fungus was first grown on 2% malt extract agar. A 5 mm^2 square was excised from the edge of the fungal colony that had been growing on 2% malt extract agar for one week at 22 °C and inoculated into autoclave sterilised (121 °C, 15 min) mushroom spawn bags (Microsac, $SacO_2$, Belgium). Each Microsac (49 cm x 22 cm with 5 aeration filter seams) containing 200 g wheat grain, 1.2 g calcium sulphate and 220 ml distilled water, were heat sealed and incubated for 3 weeks at 22 °C.

Preparation of Microsacs containing wheat straw and mineral nutrients

The substrate was prepared by soaking air dried hammermilled wheat straw that was collected through 5-10 mm screens (2 kg), in mineral solution [10 L, containing 2 g L^{-1} ammonium chloride, 0.5 g L^{-1}, magnesium sulphate, 0.2 g L^{-1} potassium dihydrogen phosphate, 0.2 g L^{-1} disodium hydrogen phosphate, 0.35 g L^{-1} manganese sulphate, 0.007 g L^{-1} ferrous sulphate, and 0.007 g L^{-1} copper sulphate] at 80 °C for 30 min. After soaking, the excess liquid was allowed to freely drain through a sieve. Half of this wheat straw was used to prepare six Microsacs (40 cm x 51 cm with 4 filter seams) each containing saturated wheat straw (250 g) and the remaining wheat straw was used to determine moisture content.

The other half of the wheat straw was placed into a large cloth bag and centrifuged at 2800 rpm for 5 min (White Knight spin dryer) to obtain desaturated wheat straw. This wheat straw was distributed as 250 g into

each of six Microsacs and moisture content was determined using the remaining wheat straw. All of the Microsacs containing the wheat straw were autoclaved at 121 °C for 60 min which was demonstrated to have no effect on the moisture content. Once the substrate had equilibrated to room temperature, each Microsac was inoculated with 45.0 ± 8.3 g of fungus infected grain to roughly equal proportions as judged by eye with the aim of preparing the substrate bags relatively quickly to avoid potential contamination issues with an open spawn bag. Then the bags were heat sealed, thoroughly mixed and weighed again to determine the weight of grain added. The Microsacs were incubated at 22 °C, 65% relative humidity for 42 days for *P. eryngii* which reflected the time required for the fungus to completely colonize the wheat straw.

Preparation of Microsacs containing wheat straw and organic nutrients

Substrate was prepared by soaking air dried milled wheat straw (2 kg) in hot water (10 L) at 80 °C for 30 min and removing the excess by sieving. Half of this wheat straw was used to prepare six Microsacs each containing 250 g wet wheat straw, 73 g dry wheat bran (purchased from a health store and <2 mm diameter) and 11 g dry calcium sulphate which was thoroughly mixed. The other half of wheat straw was centrifuged at 2800 rpm for 5 min and 250 g of this desaturated wheat straw was placed into each of six Microsacs along with 73 g dry wheat bran and 11 g dry calcium sulphate which was thoroughly mixed. Each of the Microsacs were thoroughly mixed, autoclaved and once cooled, were inoculated with 20.4 ± 4.6 fungus infected wheat grain and weighed again to determine the weight of grain added. The Microsacs incubated at 22 °C for 21 days in a humidity controlled room at 65%. The addition of wheat bran to wheat straw enabled fungal colonization by both fungi to occur more rapidly compared to the previous experiments where only wheat straw was used.

Analysis of samples

The contents of each Microsac was thoroughly mixed and samples were collected at the start and at the end of the experiment to determine mass loss, moisture content and fibre composition. Fibre analysis was determined as previously described (Baker *et al*. 2015) although the lignin content was determined by incubating each of the Ankom Microfiber bags after ADF extraction in 72% (v/v) H_2SO_4 (5 ml) for 2 h at 22 °C, then adding distilled water (140 ml) and autoclaving for 1 h at 121 °C 15 p.s.i. Finally, the bags were dried at 103 °C for 24 h and weighed. The ash content was determined in the Microfiber bags by furnace drying the bags after NDF, ADF and lignin extraction at 600 °C for 4 h. The actual hemicellulose, cellulose and lignin contents were determined by subtracting the ash content after NDF, ADF and lignin extraction, respectively.

Determination of moisture content

The moisture content was determined on triplicate samples by oven drying at 70 °C for 24 h to a constant dry weight and calculating the difference of wet weight and dry weight divided by the wet weight which was then expressed as a percentage.

Statistical analysis

Statistical analysis of the calculated data was performed using Student's *t*-test and values with $P<0.05$ are described as statistically significant as described in the results and discussion. The data presented in the tables showing statistical differences were analysed using SPSS Statistics 22 by analysis of variance..

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Conflicts of interest

None of the authors has any conflict of interest in publishing this study.

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Table 1 The moisture percentage of wheat straw inoculated with *P. erygnii* at the start and at the end when supplemented with mineral salts or with wheat bran.

	WS	WS + WB
saturated (at start)	78.7 ± 0.8 [a]	59.4 ± 0.8 [c]
saturated (at end)	75.5 ± 0.9 [ab]	63.4 ± 2.6 [c]
desaturated (at start)	67.0 ± 0.4 [bc]	44.6 ± 6.9 [d]
desaturated (at end)	67.4 ± 0.5 [bc]	46.7 ± 2.9 [d]

Means within a row lacking a common superscript differ ($P < 0.05$).

Table 2 Comparison of fungal degradation by *P. erygnii* for each of the fibre components under saturated (wet) and desaturated (dry) conditions at the start and end for wheat straw only (WS) or wheat straw with wheat bran (WS+WB). Samples were analysed on days 0 (D0), 21 (D21) and 42 (D42).

		non fibre	hemicellulose	cellulose	lignin	ash
WS	wet D0	19.6 ± 0.2 ^{a}	29.5 ± 0.5 ^{a}	41.2 ± 0.8 ^{a}	6.8 ± 0.5 ^{a}	4.1 ± 0.1 ^{a}
	wet D42	32.3 ± 3.2 ^{b}	22.2 ± 0.5 ^{b}	37.8 ± 2.7 ^{a}	4.7 ± 0.8 ^{a}	4.5 ± 0.1 ^{a}
	dry D0	19.0 ± 0.5 ^{a}	30.1 ± 1.0 ^{a}	41.3 ± 0.8 ^{a}	5.0 ± 2.6 ^{a}	3.9 ± 0.1 ^{a}
	dry D42	31.0 ± 0.1 ^{b}	21.3 ± 2.3 ^{b}	36.3 ± 4.1 ^{ab}	4.7 ± 1.5 ^{a}	4.4 ± 0.3 ^{a}
WS + WB	wet D0	38.4 ± 0.9 ^{b}	27.4 ± 0.5 ^{a}	21.3 ± 0.5 ^{c}	5.7 ± 0.8 ^{a}	7.1 ± 0.8 ^{b}
	wet D21	40.1 ± 1.8 ^{b}	21.5 ± 0.7 ^{b}	22.5 ± 1.8 ^{c}	5.4 ± 0.3 ^{a}	10.4 ± 1.0 ^{c}
	dry D0	31.6 ± 7.9 ^{b}	27.8 ± 1.6 ^{a}	24.7 ± 6.5 ^{c}	7.8 ± 1.8 ^{a}	8.1 ± 1.6 ^{b}
	dry D21	30.4 ± 3.6 ^{b}	28.3 ± 4.7 ^{a}	34.2 ± 14.7 ^{bc}	6.6 ± 0.8 ^{a}	8.2 ± 1.3 ^{b}

Means within a row lacking a common superscript differ ($P < 0.05$).

Table 3 Percentage of delignification and total lignin degradation by *P. erygnii* with respect to different fungal inocula contained within grain into wheat straw immersed in mineral salts (WS) and into wheat straw supplemented with wheat bran (WS+WB) under saturated and desaturated conditions.

		Inoculum weight (g)	Delignification (%)	Total lignin degraded (%)
WS	Saturated	41.2 ± 1.5 [a]	32.3 ± 11.5 [a]	33.3 ± 10.9 [a]
	Desaturated	48.8 ± 11.2 [a]	19.5 ± 14.4 [a]	29.4 ± 10.2 [a]
WS+WB	Saturated	16.9 ± 1.4 [b]	5.9 ± 6.1 [a]	17.9 ± 8.9 [a]
	Desaturated	23.0 ± 4.5 [b]	15.8 ± 10.5 [a]	19.8 ± 12.6 [a]

Means within a row lacking a common superscript differ ($P < 0.05$).